Towards the Discovery and Pharmacological action of Novel β-lactam Antibiotic Moieties

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MSc in Drug Chemistry

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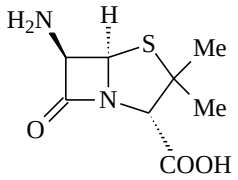
1.0 INTRODUCTION

There is a continuous need for new antibiotics to overcome antibiotic-resistant strains of organisms. However the antibiotic development has been very slow with only four new classes of antibiotics being introduced into pharmaceutical market[1]. The inevitable rise of resistance will erode the utility of today's antibiotics. To date most of the pharmaceutical research has concentrated on improving the pharmacokinetics and pharmacodynamic properties of the drug[1]



6- Aminopenicillanic acid

Penicillin contains a highly unstable bicyclic system consisting of 4 membered β-lactam ring fused to a five membered thiazolidine ring. The skeleton of the molecule is derived from the amino acids cysteine and valine[2].

The bicyclic system of penicillin goes through a large angle and torsional strains. Acid-catalysed ring opening relieves these rings by breaking open the more highly strained four-membered ring. The β-lactam of penicillin is usually more reactive toward nucleophiles than are normal amides. Substituent effects on both the acyl and amine portions of the β-lactam should be considered. For example, carbon- β-lactam nitrogen bond fission in penicillin's involves the removal of a much more weakly basic amine than that normally found in amides. A simple way to determine the reactivity of the β-lactam antibiotics is to examine their rates of hydrolysis[2].

The remarkable four-membered β-lactam ring of penicillin, which was so decisively revealed by Hodgkin's crystallographic analysis (1945), also turned out to be the motif that was responsible for the lethal action of the drug against bacteria. This activity was found to be related to the

conformation adopted by penicillin, where in the fused 4, 5-ring system enforces an orthogonal alignment of the nitrogen lone pair and the carbonyl π-bond such that the resonance stabilization exhibited by traditional amides cannot be attained in this case. This feature, in combination with the intrinsic strain of the four-membered ring, creates a situation where the carbonyl functionality of the β-lactam ring acts as a highly effective acylating agent due to its particularly strong electrophilic reactivity. Thus, it is now known that penicillin irreversibly acylates the bacterial transpeptidase enzyme responsible for the cross-linking reaction which unites the terminal glycine residue of a pentaglycine strand with the D-ala residue of a neighbouring pentapeptide, in an crucial step during the construction of bacterial cell walls. The acylation process deactivates this crosslinking enzyme, thereby compromising the integrity of the bacterial cell wall, resulting in rapid cell death. Unlike bacteria, only a phospholipid membrane surrounds mammalian cells, so transpeptidase inhibition is completely selective for bacterial cells. It has been shown that the penicillin molecule adopts an overall conformation that is very similar to the D-ala-D-ala residue of the substrate involved in this chain elongation process, thus it gains ready access to the active site of the enzyme where it reacts to disable its host[3].

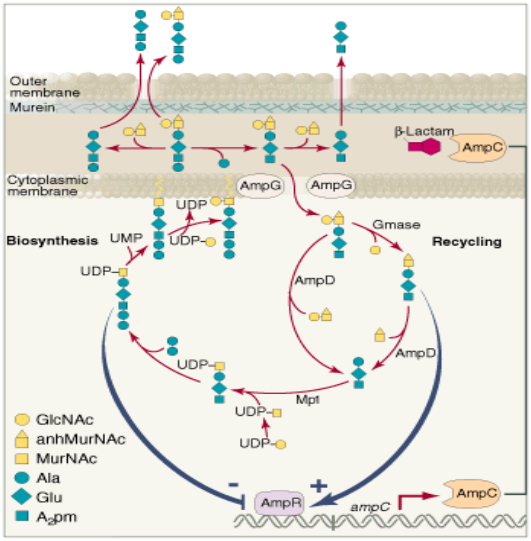


Fig 1: A model for β -lactamase induction based on sensing the relative levels of murein intermediates in the cytoplasm[5].

1.1 Molecular Models of Penicillin and of Acyl-D-alanyl-D-alanine: Penicillin is in epitome an acylated cyclic dipeptide containing the amino acids L-cysteine and D-valine. It can be viewed as an analogue of the acylated D-alanyl-D-alanine in the linear glycopeptide. The occurrence of D-iso-glutamine in the cell wall and in the nascent glycopeptide units makes it unlikely that penicillin with its free carboxyl group is an analog of L-alanyl-D-glutamic acid in the peptide chain and no evidence has been presented to support an earlier idea that penicillin is a structural analogue of NAM. In fact, the terminal D-alanine contains the only free carboxyl group in the nascent glycopeptide which might resemble that in penicillin. The atoms of the peptide chain in penicillin are fixed in position by the ring system. One of the possible conformations of the peptide chain of D-alanyl-D- alanine is almost identical to that of penicillin. The methyl group of the D-alanyl residue has a proton as its analog in the penicillin molecule[16].

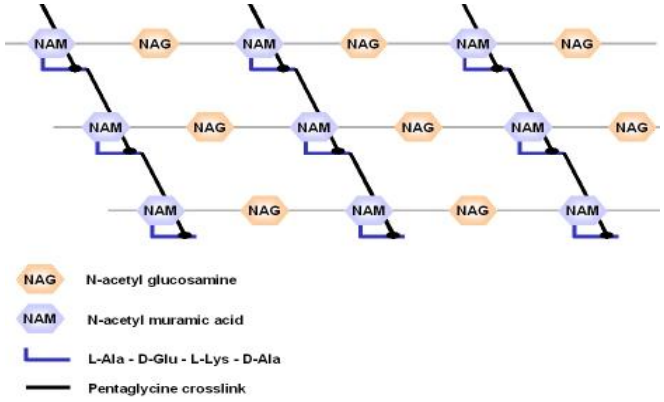


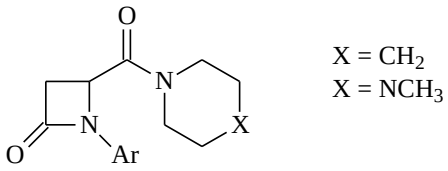
Fig 2: **Biochemistry of Bacterial Cell wall[6]**

1.2 Discovery of 6-APA: The novel penicillins were synthesized using p-amino benzyls as the starting material. The modifications were carried at the p-amino group in the β-lactam scaffold. The discovery of 6-Aminopenicillanic acid (6-APA) made a range of discovery of β-lactam antibiotics as an antibacterial agent[8].

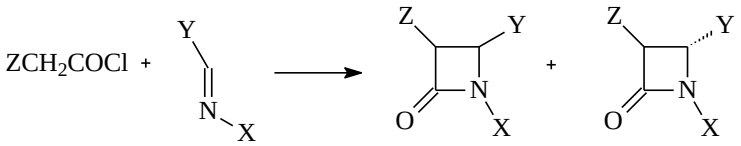
Medicinal chemists are carrying out remarkable work in the field of β-lactam chemistry, as there is a need for the potent and effective β-lactam antibiotic drugs thereby designing new functionalised

2-azetidinones. These are also used as the synthons in the preparation of variety of heterocyclic compounds.

1.3 Anticancer activity of β-lactams: The synthesis of novel β-lactams using polyaromatic imines structural units posses anticancer activity. Open chain diamides which are the conformationally restricted analogues helps to increase the potency and lower the cholesterol level in human plasma[7].



Novel β-lactam with polyaromatic imines were synthesized using Staudinger reaction. The reaction with nitrogen containing acid chlorides with diaryl imines produces cis β-lactam under Staudinger reaction[7].



The trans-isomer is formed through the isomerization of enolates, the iminium ion is stabilized at the nitrogen of the electron withdrawing polyaromatic group. This allows the rotation of the bond and results in the trans-β-lactam formation. The anticancer activity of β-lactam was assayed on human cell lines[10].

2.0 RESULTS AND DISCUSSION

Model amide reactions carried to select the appropriate method for penicillin synthesis: The starting materials were selected with similar pKa value and nucleophilicity.

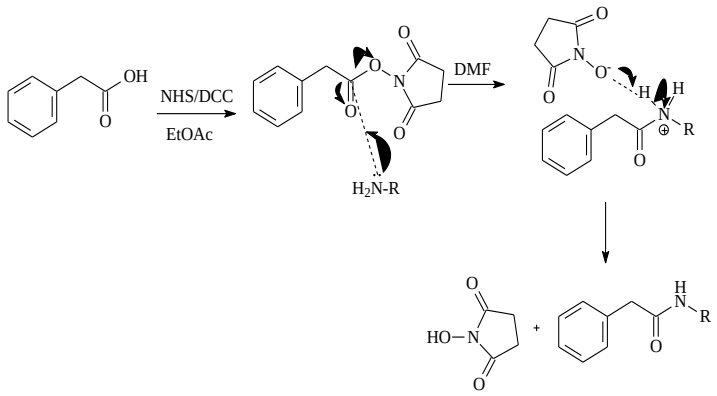
In the preparation of model amides/anilines the following methods were examined

a. Via activated ester N-Hydroxysuccinimide (NHS) / Dicyclohexylcarbodiimide (DCC)

b. Via mixed anhydride

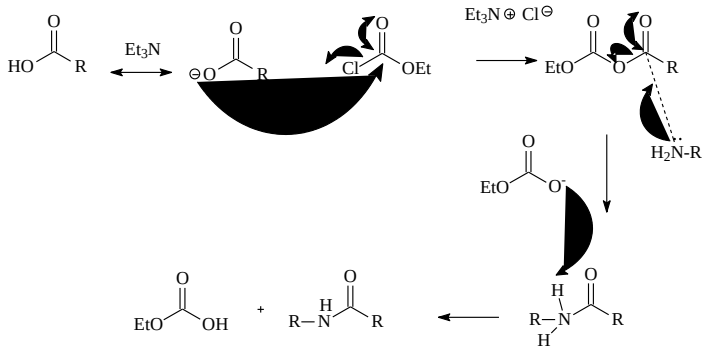
c. Using EDC (N-[3-dimethylaminopropyl]-N'-ethylcarbodiimide HCl)

Reaction Mechanisms:



Scheme: 1 (Reaction Mechanism for NHS / DCC Method)

The carbonyl carbon of the phenylacetic N-hydroxysuccinimide ester is attacked by the aniline in presence of Dimethyl formamide (DMF) to give target molecule with a NHS[10] (Scheme 1).

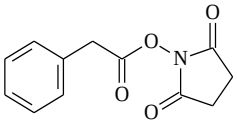
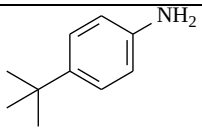
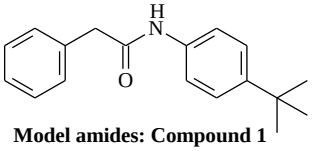
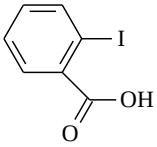
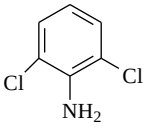
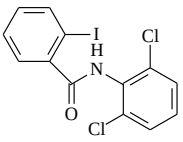
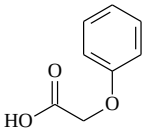
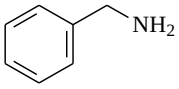
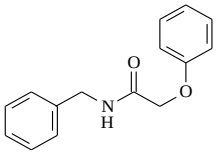


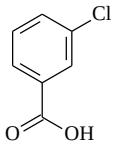
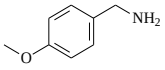
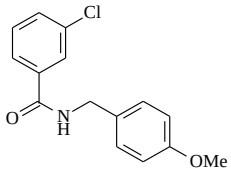
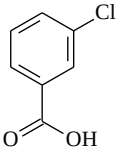
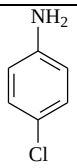
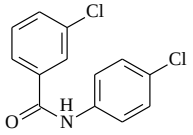
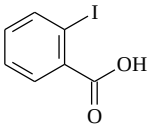
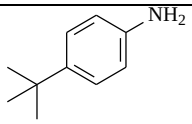
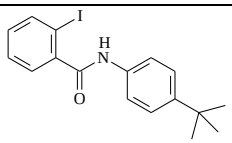
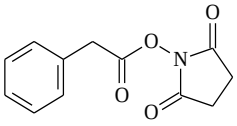
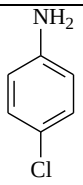
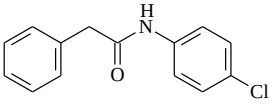
Scheme 2: (Reaction Mechanism for Mixed Anhydride Method)

The carboxylic acid derivative reacts with ethylchloroformate in presence of triethylamine as a base and Dry Dichloromethane (DCM) to give the triethylamine salt ions resulting in the formation of anhydride complex. The nucleophilic N of the amine attacks the carbonyl carbon of the carboxylic acid forming the amide and acid[12].

SCHEME TABLE FOR MODEL AMIDES & ANILINE PREPARATIONS

(Table : 1)

Sl. No	Method used	Starting material	Amines and Anilines	Product	% yield
★ 1.	Activated ester (1 step)	 **Phenylacetic N-hydroxysuccinimide ester**	 **4-tert-Butylaniline**	 **Model amides: Compound 1**	50%
2 ★.	NHS Activated Ester (Step 2)	 **2-Iodobenzoic acid**	 **2,6 Dichloroaniline**	 **Model amide: Compound 2**	i. 100% ii. 18%
3.	Mixed Anhydride	 **Phenoylacetic acid**	 **Benzylamine**	 **Model amide: Compound 3**	53%

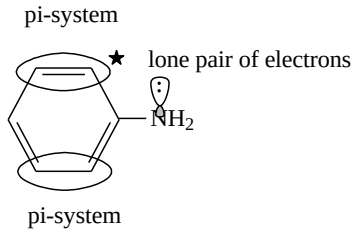
4.	NHS activated ester Method (Step 2)	 **3-Chlorobenzoic acid**	 **4 - Methoxybenzylamine**	 **Model amides: Compound 4**	i. 100% ii. 18%
5.	Mixed Anhydride	 **3-Chlorobenzoic acid**	 **4-Chloroaniline**	 **Model amides: Compound 5**	16%
6.	Mixed Anhydride	 **2-Iodobenzoic acid**	 **4-tert-Butylaniline**	 **Model amides: Compound 6**	4%
7.	NHS activated ester Method (Step 1)	 **Phenylacetic N-hydroxysuccinimide ester**	 **4-Chloroaniline**	 **Model amides: Compound 7**	53%

2.1 NHS/DCC Method: Reactions were initially carried out at room temperature with Phenylacetic N-hydroxysuccinimide ester was coupled with 4-*tert*-butylaniline and 4-Chloroaniline to form **Compound 1** and **Compound 7** with yields of 50% and 53% respectively. A modification by heating the reaction mixture at 100ºC for 2 h gave an amide product[11].

The formation of the amide in **Compound 1** was confirmed by the distinctive broad singlet peak in the ^{1}H NMR at δ 6.99 ppm (Appendix figure 3). IR and LC-MS was consequently used to fully exemplify the amide. The formation of the amide in **Compound 7** was confirmed by the distinctive broad singlet peak in the ^{1}H NMR at δ 7.10 ppm (Appendix figure 14). IR and LC-MS was consequently used to fully exemplify the amide (See Appendix figure 5). Substituent on the side ring of anilines has no apparent effect.

The same method replacing acids as 2-Iodobenzoic acid and 3-Chlorobenzoic acid was coupled to form **Compound 2** and **Compound 4** with equal yield of 18%. Amide product obtained by this method resulted in a less yield it was then followed by flash chromatography[13]. The amine/anilines don't appear to have an effect.

This problem is due to the presence of chlorine molecules in 2^{nd} and 6^{th} position of the aromatic ring. The lone pair of electrons on the Anilines conjugates the π-system of the aromatic ring and makes the π- system electron rich. The chlorine on the aromatic ring pulls the electrons of p-orbitals out of the π-system and makes π-system electron deficient. And therefore the lone pair of electrons of the nitrogen fails to attack the carbonyl carbon.



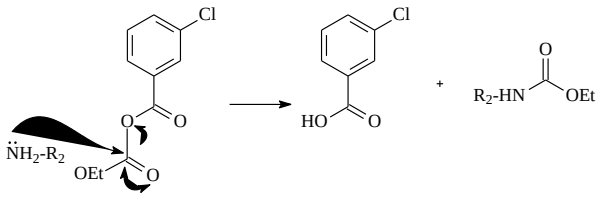
The ability of reactivity of the anilines in a sequence of the reaction was difficult and that this was mainly due to the reduced nucleophilicity of the aniline nitrogen. The poor reactivity of the anilines is due to the increased conjugation of the lone pairs present on the nitrogen molecule with the π system of the aromatic ring. This was driven by the increased electron deficiency caused by the electron withdrawal by the chlorine substituents on the aromatic ring.

Preparation of compound 2 and 4 proved to be successful and carrying out the reactions overnight resulted in the formation of the amides were confirmed by ^{1}H-NMR that resulted in the characteristic broad singlet peaks at δ 5.39 ppm and δ 6.59 ppm (Appendix figure 6 and 8) respectively.

2.2 Mixed Anhydride method: Another pilot method scrutinized was activation of the chosen acid by the formation anhydride by coupling with ethyl chloroformate followed by direct addition of the

amine/aniline to give the resulting amide (Scheme 2). Attack by the anilines or amines take place at the acid carbonyl with loss of the ethoxy carboxylate. This was due to electron donation to the chloroformate carbonyl by both of its oxygen substituents. Thereby stabilising the carbonyl carbon and reducing its electrophilicity and directing the attack at the highly reactive carbonyl group of an acid[12].

Compounds 3, 5 and 6 were prepared using the mixed anhydride method by the activation of the acid by ethyl chloroformate. Compounds 3, 5 and 6 were produced in 53%, 16% and 4% respectively. In the ^{1}H-NMR confirming the formation of the amide in the above mentioned compounds show a characteristic broad singlet peak 4.99 ppm (Compound 3), 6.58 ppm (Compound 5) and 6.58 ppm (Compound 6) see (Appendix figure 7, 12, 13).



Scheme 3: Proposed Mechanism for the formation of Ethoxy amides using Ethylchloroformate in Mixed Anhydride method

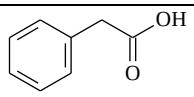
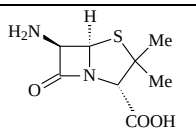
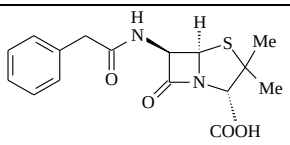
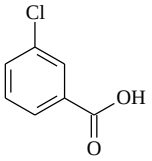
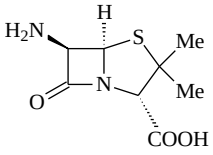
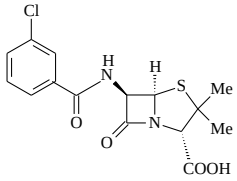
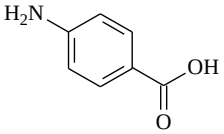
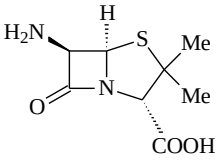
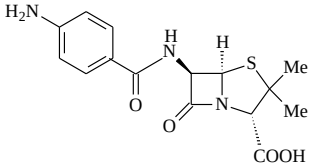
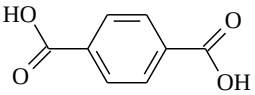
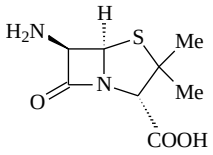
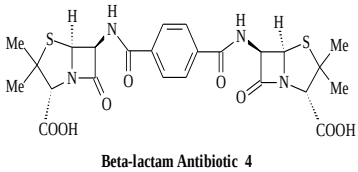
Formation of the ethoxy amide is the reduced reactivity of the acid carbonyl by increased conjugation with the adjacent π system of the aromatic ring. The chlorine molecule in the *ortho* position of the chosen aromatic acid has an electron withdrawing effect on the ring increasing the electron deficiency of the aromatic system. This increases the conjugation between the aromatic ring and the directly attached carbonyl reducing its reactivity and susceptibility to nucleophilic attack from the aniline/amides. The chlorine substituent on the aromatic acid may also provide some steric hindrance further reducing the vulnerability of the carboxylic acid carbonyl to nucleophilic attack. Due to this feature to decreases the difference in reactivity of the carbonyl carbon of the

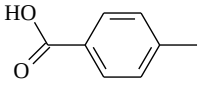
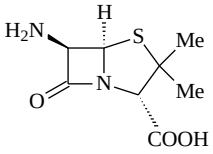
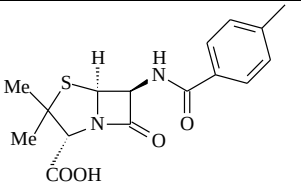
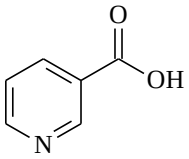
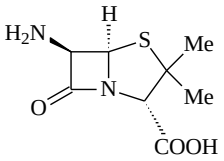
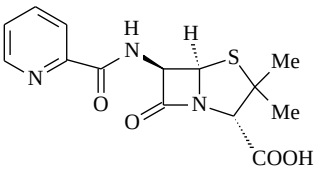
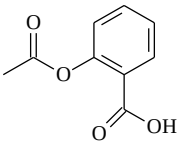
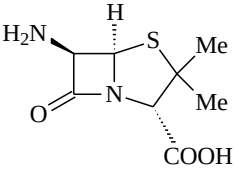
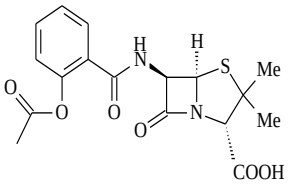
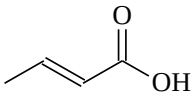
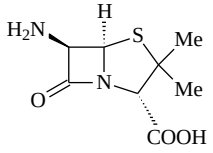
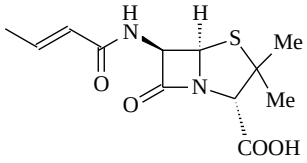
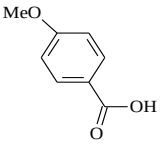
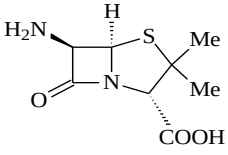
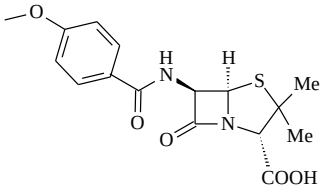
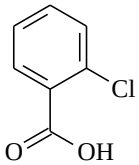
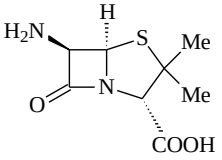
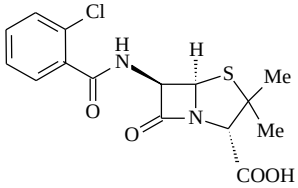
aromatic acid and the ethoxy carbonyl carbon that allows attack leading the ethoxy amide as a major product.

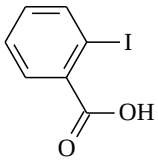
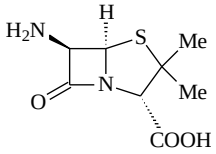
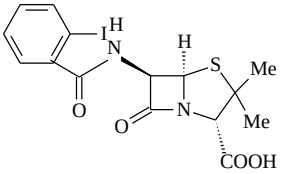
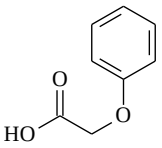
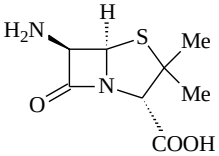
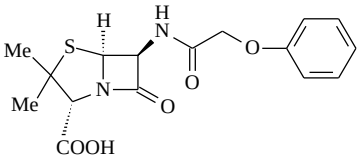
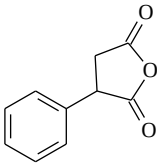
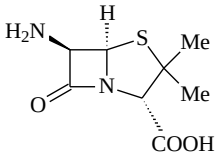
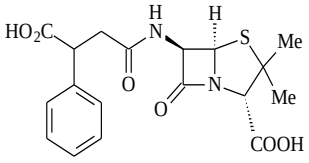
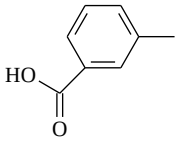
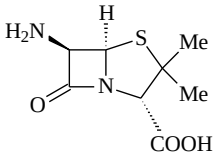
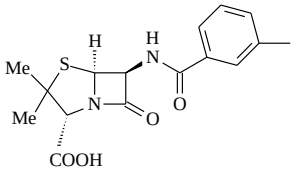
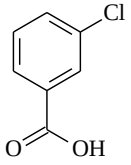
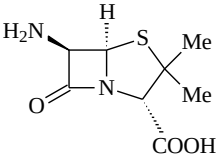
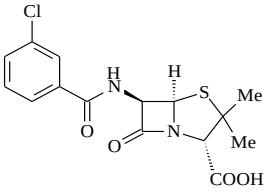
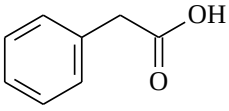
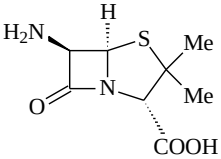
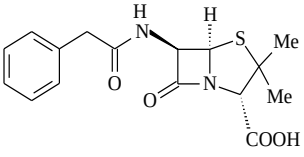
Although the formation of the ethoxy amide, it was made certain that the mixed anhydride method was the most suited for the coupling of aromatic acids with 6-APA compared to the DCC / NHS method. Mixed anhydride method was selected to synthesize the β-lactam derivatives as it was proved by the model amide reactions.

SCHEME TABLE FOR THE PREPARATION OF β-LACTAM ANTIBIOTIC MOIETIES

(Table : 2)

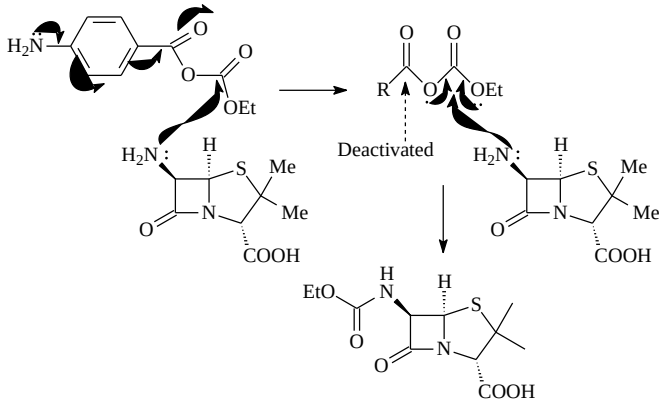
Sl. No	Method used	Starting material	6-Aminopenicillanic acid	Product	% yield
1.	Mixed Anhydride	 **Phenylacetic acid**	 **6-APA**	 **Beta-lactam Antibiotic 1**	9%
2.	Mixed Anhydride	 **3-Chlorobenzoic acid**	 **6-APA**	 **Beta-lactam Antibiotic 2**	100%
3.	Mixed Anhydride	 **4-Aminobenzoic acid**	 **6-APA**	 **Beta-lactam Antibiotic 3**	100%
4.	Mixed Anhydride	 **Terephthalic acid**	 **6-APA**	 **Beta-lactam Antibiotic 4**	40%

5.	Mixed Anhydride	 **p-Toluic acid**	 **6-APA**	 **Beta-lactam Antibiotic 5**	40%
6.	Mixed Anhydride	 **Nicotinic acid**	 **6-APA**	 **Beta-lactam Antibiotic - 6**	20%
★ 7.	Mixed Anhydride	 **Aspirin (Acetyl Salicylic acid)**	 **6-APA**	 **Beta-lactam Antibiotic - 7**	70%
8.	Mixed Anhydride	 **Crotonic acid**	 **6-APA**	 **Beta-lactam Antibiotic - 8**	65%
9.	Mixed Anhydride	 **p-Anisic acid**	 **6-APA**	 **Beta-lactam Antibiotic - 9**	65%
10.	Mixed Anhydride	 **2-Chlorobenzoic acid**	 **6-APA**	 **Beta-lactam Antibiotic - 10**	8%

11.	Mixed Anhydride	 **2-Iodobenzoic acid**	 **6-APA**	 **Beta-lactam Antibiotic - 11**	25%
★ 12.	Mixed Anhydride	 **Phenoxyacetic acid**	 **6-APA**	 **Beta-lactam Antibiotic - 12**	75%
★ 13.	NHS Method	 **Phenylsuccinic anhydride**	 **6-APA**	 **Beta-lactam Antibiotic - 13**	-
★ 14.	Mixed Anhydride	 **m-Toluic acid**	 **6-APA**	 **Beta-lactam Antibiotic - 14**	20%
15.	DCC Method	 **3-Chlorobenzoic acid**	 **6-APA**	 **Beta-lactam Antibiotic - 15**	2%
16.	Mixed Anhydride	 **Phenylacetic acid**	 **6-APA**	 **Beta-lactam Antibiotic - 16**	30%

2.3 Synthesis of β-lactam moieties: β-lactam Antibiotic 1 (Phenylacetic 6 Amino Penicillanic acid) was synthesized using mixed anhydride method using ethylchloroformate in presence of triethylamine as a base gave a yellow colored solution which was further worked up to get the Potassium salt of Penicillin[12]. As the penicillin is dissolved in methanol, product obtained was dissolved in Methanol and Ether which precipitates out the inorganic salt was discarded and the dissolved mixture was subjected to evaporation under vacuum pressure. The obtained product was hygroscopic that it would turn to oil when exposed to air. The obtained product was dried using dry Acetone or Petrol 40-60. The obtained product was crystal pale yellow solid. A characteristic doublet peaks at δ 5.25 and 5.45ppm of β-lactam protons was observed in the ^{1}H NMR confirming the formation of the β-lactam protons in the product obtained See Appendix (Figure 15 and 16)

β-lactam Antibiotic 2 (3-Chlorobenzoic 6-Amino Penicillanic acid) was not successfully isolated, the obtained product resulted in the starting material due to the presence of chlorine in the *ortho* position as discussed earlier in the model amide reactions. Due to the successful coupling of 6-APA with phenylacetic acid, the further synthesis was focused on aminobenzoic acids, 4 aminobenzoic acid was used to couple with 6 APA which resulted in the ethoxy penicillin (**β-lactam Antibiotic 3**) because the ethylchloroformate is deactivated and forms the ethoxy penicillin. (See Appendix figure 17 and 18).



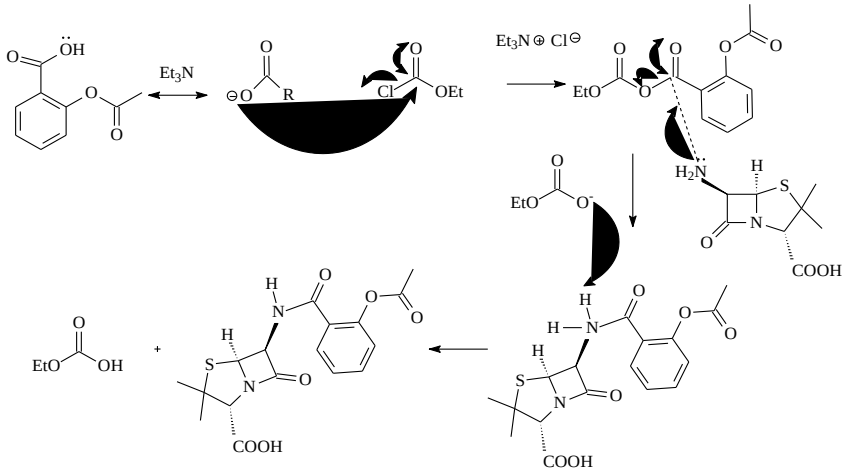
Ethoxy Penicillin

Scheme 4: Proposed mechanism for the formation of Ethoxy Penicillins

An attempt was made in coupling diacids like Terephthalic acid with 6 APA but the **(β-lactam Antibiotic 4**) was unsuccessful with synthesizing the starting material.

Synthesis of **β-lactam Antibiotic 5** (p-Toluic 6 amino penicillanic acid) by coupling of p-Toluic acid to 6 APA via mixed anhydride resulted in a total yield of 40%. A characteristic doublet peaks was observed at δ 4.90ppm and 5.20ppm for the β-lactam-proton moieties respectively See Appendix (Figure 19 and 20). A medium N-H Stretch was observed in 3371.327 cm^{-1} and a strong β-lactam stretch at 1770.493 cm^{-1} was observed with respect to IR spectrum.

With the satisfactory results with **(β-lactam Antibiotic 6)**, further synthesis was focused on the carboxylic acids with the pyridine ring in a 2 mmol scale. Nicotinic acid and 6 APA was coupled via mixed anhydride, where the results were quite promising. A characteristic doublet peaks was noted at δ 4.10, 5.25 and 5.45ppm holding the β-lactam proton peaks (See Appendix figure 21 and 22). A medium stretch at was noted at 3321.112 cm^{-1} which gives a promising amide stretch with respect to IR spectrum.



Scheme 5: Proposed Reaction Mechanism for β-lactam Antibiotic 7

Further synthesis was to couple the drugs with antipyretic and antibiotic moieties. **(β-lactam Antibiotic 7)** was the novel product as it was identified by the database reaxys and

scifinder. Acetyl salicylic acid an antipyretic drug and antiplatelet drug which is used in cardiovascular treatment was coupled with 6-APA gave an excellent result with a yield of 70%. A characteristic peak was observed in ^{1}H NMR; doublets peaks at δ 4.10, 5.25 and 5.45ppm assigning β-lactam protons and an ArH multiplets peak at δ 6.51-7.71 (See Appendix figure 23 and 24). ^{13}C NMR was gave a confirmation of the carbon atoms of β-lactam ring at 75.1720 (C^{16}) and thiazolidine ring at 67.1438 (C^{19}). (See appendix figure 25). LC-MS of the compound confirmed the formation of the Acetyl salicylic penicillin and gave $[M-H]^-$ peak at 377.00 m/z (See Appendix figure 26) calculated molecular weight from exact isotopic masses being 378.0885 g/mol.

An attempt was made in coupling butenoic acids like Crotonic acid with 6 APA but the **(β-lactam Antibiotic 8)** was unsuccessful with synthesizing the starting material. Ethyl chloroformate failed to form the penicillins. Slight modification was made in the procedure by using isobutyl chloroformate due to less steric hindrance.

Further synthesis was made in coupling methoxy acids by coupling p-Anisic acid with 6 APA, using iso-butylchloroformate but the **(β-lactam Antibiotic 9**) was unsuccessful with synthesizing the starting material.

Synthesis was carried out with chloro-benzoic acids like 2-Chlorobenzoic acid with 6 APA but the **(β-lactam Antibiotic 10**) was unsuccessful with synthesizing the starting material. The NH_2 didn't react with the acid due to the conjugated π-aromatic system.

Synthesis was continued with Iodo-benzoic acids like 2-Iodobenzoic acid with 6 APA but the (**β-lactam Antibiotic 11**) was unsuccessful with synthesizing the starting material.

Phenoxyacetic acid was a novel compound as it was identified by the database reaxys and scifinder chosen as the acid to couple with 6-APA to form a phenoxy-6-APA derivative because the positioning of the acid carbonyl group. It is not directly adjacent to the aromatic ring and would therefore doesn't form ethoxy penicillins.

The **(β-lactam Antibiotic 12)** was synthesised as a fine white crystalline solid yielding 75 % of the product. The ^{1}H-NMR exhibit two doublets at δ 4.60, and 5.38 ppm that is characteristic of the β-lactam protons (Appendix figure 27 and 28). 13C displayed δ_C 27.21 ($C^{19}$and C^{20}) of CH_3 groups and 74.11 (C^{16}) carbon of the thiazolidine ring, 59.65 (C^{12}), 68.08 (C^{14}), 174.75 (C^{13}) carbons of β-lactam ring (Appendix figure 29). LC-MS of the compound gave a peak at m/z 348.95 $[M-H]^-$ (Appendix figure 30) calculated mass being 350.093 g / mol.

An attempt was made in coupling activated Phenylsuccinic anhydride acid with 6 APA using DMAP as a catalyst which makes 6 APA more nucleophilic but the **(β-lactam Antibiotic 13)** was unsuccessful with synthesizing the starting material.

Further synthesis was to couple m-Toluic acid to 6 APA using iso-butylchloroformate. (**β-lactam Antibiotic 14**) was the novel product as it was identified by the database reaxys and scifinder. m-Toluic acid was coupled with 6-APA gave an excellent result with a yield of 20%. A characteristic peak was observed in ^{1}H NMR; doublets peaks at δ 5.10, 5.25ppm assigning β-lactam protons and an multiplets peak of 4 aromatic protons at δ 7.20-7.70 (See Appendix figure 31 and 32). LC-MS of the compound confirmed the formation of the m-Toluic penicillin moieties and gave $[M-H]^-$ peak at 333.00 m/z (See Appendix 2.14b) calculated molecular weight from exact isotopic masses being 334.0984 g/mol.

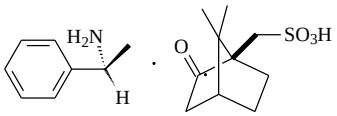
(**β-lactam Antibiotic 15)** was synthesized by coupling 3-Chlorobenzoic acid to 6 APA using NHS in presence of DMAP as a catalyst via DCC method gave a brown colored solution which was further worked up to get the Potassium salt of Penicillin. As the penicillin is dissolved in methanol, product obtained was dissolved in Methanol and Ether which helps precipitate inorganic salt was discarded and the dissolved mixture was subjected to evaporation under vacuum pressure[11]. A characteristic peaks at 3322.149 (m, N-H Stretch), 1627.678 (s, C=O Stretch), 2930.351 (m, C-H

Stretch), 1770.056 (s, β-lactam Stretch), 806.957 (s, C-Cl Stretch) cm^{-1}. The yield was not sufficient to analyze ^{1}HNMR, it gave noisy spectrum.

Further synthesis was focused to couple Phenylacetic acid to 6 APA using iso-butylchloroformate. This (**β-lactam Antibiotic 16)** Phenylacetic acid was coupled with 6-APA gave an excellent result with a yield of 30%. Isolation of Penicillin was unsuccessful, ^{1}HNMR revealed the isolation of starting material.

2.4 Preparation of racemic 2-Phenylglycine with (1*S*)-(+)-Camphor-10-sulfonic acid.

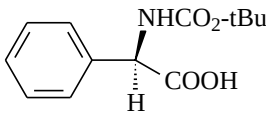
This preparation was carried out to couple the aminoacid to penicillin using D-Phenylglycine (D-PG) which is used as organic material for the synthesis of β-lactam antibiotic drugs by the preparation of racemic 2-Phenylglycine using 1(*S*)-(+)-camphor-10 sulfonic acid as the resolving agent there by seeding fine D-PG.(+)-CS crystals which resulted in a good yield[13]. The measured specific rotation was $[\alpha]_D^{20}$ = -38 and the optical purity is 80% The result obtained show 6% difference, where the optical purity with respect to the reference sample is 86.0% and specific rotation $[\alpha]_D^{20}$ = - 40.8



D-PG **(+)-CS**

2.5 Conversion of (R)-2-Phenylglycine to its BOC Derivative

The BOC derivative is prepared to protect the $-NH_2$. This was carried out by the conversion of (*R*)-2-Phenylglycine to its N-*tert*-butyloxycarbonyl (BOC) derivatives to couple with the 6-Aminopenicillanic acid;



1st crop of (*R*)-N-*tert*-butyoxycarbonyl-2-PG resulted in a good promising NMR but due to time constrain couldn't couple with my penicillin derivatives with the BOC derivatives[15]. The ^{1}HNMR shot up the peaks of ^{t}Bu at 1.45ppm, N-H peak at 7.41 and the ArH at 7.39. IR revealed a medium N-H stretch at 3377.308, strong C=O stretch at 1682.806 and medium C-H stretch at 2981.109cm^{-1}

Due to constrain of time, we couldn't work on the deprotection of penicillins using BOC derivatives, HCl deprotection and TFA deprotection.

2.6 Hydrolysis of Benzyl Penicillin and β-lactam Antibiotic 12

β-lactam drugs undergo acid-catalysed hydrolysis when administered orally which inactivates the antibiotics. The hydrolysis pathway was explicated by scanning the antibiotic samples using UV-Vis Spectrophotometer[16].

a. Calculation for Benzyl Penicillin at 240nm (Appendix 3.1a)

% Yield of the Final Product II = 27%

% Yield of the Final Product III = 4%

% Yield of the Final Product IV = 69%

b. Calculation for Benzyl Penicillin at 290nm (Appendix 3.b)

% Yield of the Final Product III = 4%

3.0 EXPERIMENTAL

The primary sources of chemicals were from Aldrich Chemical Company and Lancaster Chemicals. Chemicals were used as supplied without further purification, unless otherwise stated. Nitrogen atmosphere along with oven dried glassware were used for all the reactions requiring inert conditions. AnalaR or Laboratory grade were used for all the solvents. Petrol refers to the fraction of petroleum spirit with a boiling range of 40-60ºC.

Safety and discarding of waste aspects were maintained in accordance with the departmental rules and in compliance with COSHH regulations.

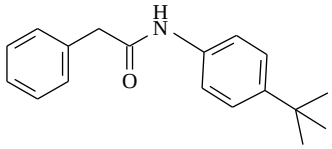
^{1}HNMR (Nuclear Magnetic Resonance) was recorded on Bruker 300WB (300MHz) spectrometer. Residual proton signals from deuterated solvents were used as references [$CDCl_3$ (7.25ppm) and Deuterium Oxide (4.70 ppm) for all other preparations]. ^{13}C NMR spectra was recorded on a Bruker 300WB (75 MHz), with Deuterium Oxide as a reference solvent. Elemental analysis was tested on a Carlo Erba 1108 Elemental Analyser controlled with CE Eager 200 software. Samples prepared for analysis were submitted either to Dr. Zuleyka McMillan or Dr. Carl Young (Post-Doc Student) or Dr. Pedro (Post-Doc Student).

LC-MS was performed with a Waters LCT Premier mass spectrophotometer consisting of an Acquity sample manager, Acquity solvent manager, Waters reagent manager and Waters 2996 photo diode array detector in electrospray ionization negative ion mode.

Thin Layer Chromatography (TLC) was performed on pre-coated plates with Kieselgel $60F_{254}$ silica gel. UV fluorescence and Ninhydrin reagent (0.3% N-Butanol + 3% Acetic acid) was used for visualization. Column chromatography was performed on Kieselgel $60F_{254}$ silica gel with eluting system given in volume: volume (Petrol 40-60: Ethyl acetate)

3.1 PREPARATION OF MODEL AMIDES

3.1(a) Preparation of model amide: coupling of Phenylacetic acid to 4-*tert* butyl aniline via NHS/DCC Method



Model amides: Compound 1

$C_{18}H_{21}NO$

To Phenyl acetic N-hydroxysuccinimide ester (1mmol, 0.23g) nitrogen gas source is supplied using micro syringe fitted to rubber tubes and add 8 mL of DMF was added and stirred about 10 min and 4-*tert* butyl aniline (1.1mmol, 0.175 mL) was added using a micro syringe as it is an irritant and the stirring is continued overnight at room temperature. The solution turned to orange.

Ethyl acetate (40 mL) is added and the solution is quenched with NaCl and AcOH solution and made up to 80 mL and was stirred for 30 min. Separation was done using separating funnel, the organic layer was washed with 8% aqueous $NaHCO_3$ (80 mL) and saturated brine solution (50 mL)

and the reaction mixture was filtered using Buchner filter and the obtained solution was transferred into a pre-weighed round bottom flask and the solution was evaporated through rotary evaporator[11].

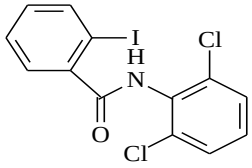
^{1}H NMR (300MHz, $CDCl_3$): δ_H 1.35 (s, 9H, ^{t}Bu), 3.75 (s, 2H, CH_2), 6.99 (s, 1H, NH), 7.28-7.45(m, 8H, ArH)

^{13}C NMR (75MHz, $CDCl_3$): δ_C 31.87 ($C^{18}$and C^{20}), 34.69 (C^{17}), 45.19 (C^{7}), 120.15(C^{12}-C^{13}), 127.86 (C^{3}, C^{16}, C^{14}), 129.79 (C^{1} and C^{5}), 135.49 (C^{11} and C^{6}), 147.94 (C^{15}), 169.24 (C^{8}).

IR: vcm^{-1} 3291.639 (m, N-H Stretch), 1655.778 (s, C=O Stretch), 2962.970 (m, C-H Stretch) cm^{-1}

LC-MS (ES^-): Calculated mass- 267.162 formula ($C_{18}H_{21}NO$) m/z 268.05 (19.7%, $M+H^+$).

3.1(b) Preparation of model amide: Coupling of 2-Iodobenzoic acid to 2,6 Dichloroaniline via DCC method



Model amide: Compound 2

$C_{14}H_{12}NOCl$

The method for the formation of the NHS ester of different Acid Derivatives was carried out as follows; To a stirred solution of 2 Iodobenzoic acid (3.11 mmol, 0.77 g), N-hydroxysuccinimide of (3.42 mmol, 0.394 g) was added and stirred with 20 mL of ethyl acetate at 0ºC and 0.706 g of Dicyclohexylcarbodimide (DCC) was added and is stirred overnight and allowed to cool at room temperature.

The resulted suspension was filtered through celite and the filtrate concentrated to afford to 2-Iodobenzoic N-hydroxysuccinimide ester (1.064 g). 2-Iodobenzoic-N-hydroxysuccinimide ester (0.377 g) is added and the flask is subjected to Nitrogen gas for drying for about 5 min. DMF is also dried using nitrogen gas using microlitre syringes and needles. DMF (8 mL) is added into the round

bottom flask containing ester using syringe and finally 2, 6 Dichloroaniline (1.1 mmol, 0.178 g) is added to the reaction mixture and the reaction mixture is stirred overnight at rt.

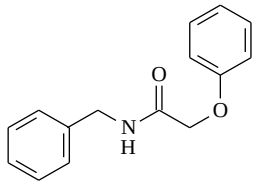
. The resulting reaction mixture is worked up as in procedure 3.1(a).

^{1}H NMR (300MHz, $CDCl_3$): δ_H 5.39 (s, 1H, NH), 7.18-8.15 (7H, m, ArH)

IR: vcm^{-1} 3356.287 (m, N-H Stretch), 1726.225 (s, C=O Stretch), 2935.906 (m, C=H Stretch), 731.723 (s, C-Cl Stretch), 519.927 (s, C-I Stretch) cm^{-1}

R_f: 0.38 (Petrol 40-60: Ethyl acetate).

3.1 (c) Preparation of Model amide formations: Coupling of phenoxyacetic acid to benzyl amine via mixed anhydride.



Model amide: Compound 3

$C_{15}H_{15}NO_2$

Dry DCM (5 mL) dissolve of triethylamine (4mmol, 0.4 mL) in a 100 mL round bottom flask and subject it to ice bath for curing about 5-10 min. Add Phenoxyacetic acid (4mmol, 0.608 g) is added at -10ºC. Ethylchloroformate (4mmol, 0.38 mL) is added dropwise and the reaction mixture is stirred for about 20 min and crystal precipitate was observed.

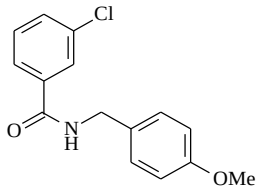
Benzylamine (4mmol, 0.436 mL) and 5 mL of dry DCM is added to dissolve the amine and the dissolved amine is added to the acid reaction mixture and the reaction mixture is stirred overnight on ice bath. The resultant mixture us washed with DCM and followed by $NaHCO_3$ (80 mL) of solution, as the DCM settles in the bottom then lower is collected for the further washing and the top layer is discarded. The reaction mixture is washed with 2x25 mL of water and further by 40 mL of 1M HCl solution and then with saturated brine (50 mL). The obtained DCM layer is dried with

Magnesium Sulphate and the solution is left for about 1h and is filtered through filter paper and the filtrate is evaporated using rotary evaporator[12].

^{1}H NMR (300MHz, $CDCl_3$): δ_H 4.40 (s, 4H, CH_2), 4.99 (s, 1H, NH), 6.92-7.39 (10H, m, ArH)

IR: vcm^{-1} 3277.792 (m, N-H Stretch), 1664.497 (s, C=O Stretch), 2935.906 (m, C-H Stretch), 1245.269 (s, C-O Stretch) cm^{-1}

3.1 (d) Preparation of model amides: Coupling of 3-Chlorobenzoic acid to 4-Methoxybenzylamine via DCC method



Model amides: Compound 4

$C_{15}H_{14}NOCl$

The method for the formation of the NHS ester of different Acid Derivatives was carried out as follows; To a stirred solution of 3-Chlorobenzoic acid (3.11 mmol, 0.485 g) and N-hydroxysuccinimide of (3.42 mmol, 0.394 g) was added and stirred with 20 mL of ethyl acetate at 0ºC and 0.706g of Dicyclohexylcarbodimide (DCC) was added and is stirred overnight and allowed to cool at room temperature.

The resulted suspension was filtered through celite by making a bed of celite on a silica sintered funnel and pour down the suspension into the silica sintered funnel and wash with ethyl acetate and subject for evaporation with rotary evaporation that afford to yield 3-Chlorobenzoic N-hydroxysuccinimide ester (0.864 g).

3-Chlorobenzoic-N-hydroxysuccinimide ester (0.271) g is subjected to Nitrogen gas for drying for about 5 min. DMF is also dried using nitrogen gas using microlitre syringes and needles. DMF (8 mL) is added into the round bottom flask containing ester using syringe and finally 4 Methoxybenzylamine (1.1mmol, 0.151g) is added to the reaction mixture and the reaction mixture is stirred overnight at room temperature[11].

The resulting reaction mixture is worked up as in procedure 3.1 (a)

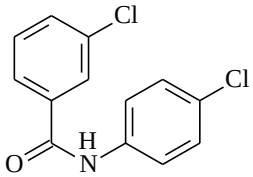
^{1}H NMR (300MHz, $CDCl_3$): δ_H 3.81 (s, 3H, CH_3), 4.56 (s, 2H, CH_2), 6.59 (s, 1H, NH), 6.87-7.78 (8H, m, ArH)

^{13}C NMR (75MHz, $CDCl_3$): δ_C 43.5336 (C^{11}), 55.2616 (C^{19}), 114.1181 ($C^{17}$and C^{15}), 125.0352(C^{5}), 129.8216 (C^{2}, C^{12}, C^{13} and C^{14}), 131.4520 (C^{1}), 134.6556 (C^{6}), 136.1621 (C^{4}), 159.1118(C^{16}), 165.8623 (C^{8}).

IR: vcm^{-1} 3270.988 (m, N-H Stretch), 1740.648 (s, C=O Stretch), 2941.886 (m, C-H Stretch), 1240.595(s, C-O Stretch), 693.020 (s, C-Cl Stretch) cm^{-1}

LC-MS (ES^-): Calculated mass – 275.5550 formula ($C_{15}H_{14}NClO_2$) m/z 275.90 (100%, $M+H)^+$, 273.90 (100%, $M-H)^-$

3.1 (e) Preparation of Model amides: Coupling of 3-Chlorobenzoic acid to 4-Chloroaniline via Mixed Anhydride method



Model amides: Compound 5

$C_{13}H_9NOCl_2$

Dry DCM (5 mL) of dissolve of triethylamine (4mmol, 0.4 mL) in a 100 mL round bottom flask and subject it to ice bath for curing about 5-10 min. Add 3-Chlorobenzoic acid (4mmol, 0.626g) is added at -10ºC. Ethylchloroformate (4mmol, 0.38 mL) is added dropwise and the reaction mixture is stirred for about 20 min and crystal precipitate was observed.

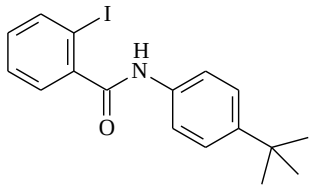
4-Chloroaniline (4mmol, 0.510 g) was added into a 100 mL separate round bottom flask and about 5 mL of dry DCM is added to dissolve the amine and the dissolved amine is added to the acid reaction mixture and the reaction mixture is stirred overnight on ice bath. The workup is carried out as same as in procedure[12] 3.1 (c)

Physical data: Pale pink solid

^{1}H NMR (300MHz, $CDCl_3$): δ_H 6.58 (s, 1H, NH), 7.26-7.76 (8H, m, ArH)

IR: vcm^{-1} 3350.034 (m, N-H Stretch), 1650.719 (s, C=O Stretch), 3128.452 (m, C-H Stretch), 641.368 (s, C-Cl Stretch) cm^{-1}

3.1 (f) Preparation of Model amides: Coupling of 2-Iodobenzoic acid to 4-*tert*-butylaniline via mixed anhydride



Model amides: Compound 6

$C_{17}H_{18}NOI$

Dry DCM (5 mL) dissolve of triethylamine (4 mmol, 0.4 mL) in a 100 mL round bottom flask and subject it to ice bath for curing about 5-10 min. Add 2-Iodoobenzoic acid (4 mmol, 0.992 g) is added at -10ºC. Ethylchloroformate (4 mmol, 0.38 mL) is added dropwise and the reaction mixture is stirred for about 20 min and crystal precipitate was observed.

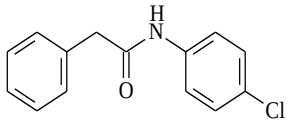
4-*tert*-butylaniline (4mmol, 0.596 g) was added with dry DCM (5 mL) is added to dissolve the amine and the dissolved amine is added to the acid reaction mixture and the reaction mixture is stirred overnight on ice bath. The workup is carried out as same as in procedure[12] 3.1(c)

Physical data: Pale yellow solid

^{1}H NMR (300MHz, $CDCl_3$): δ_H 1.35 (s, 3H, CH_3), 6.58 (s, 1H, NH), 7.15-7.95 (8H, m, ArH)

IR: vcm^{-1} 3276.006 (m, N-H Stretch), 1655.458 (s, C=O Stretch), 3038.169 (m, C-H Stretch) cm^{-1}

3.1 (g) Preparation of model amides: Coupling of Phenyl acetic N- hydroxysuccinimide ester to 4-Chloroaniline via DCC method



Model amides: Compound 7

$C_{14}H_{12}NOCl$

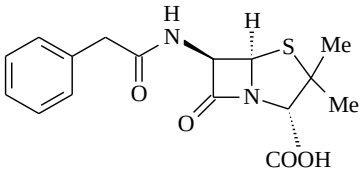
Phenyl acetic N-hydroxysuccinimide ester (1mmol, 0.23g) is added to 50cm^3 of round bottom flask and nitrogen gas source is supplied using micro syringe fitted to rubber tubes and add 8 mL of DMF was added and stirred about 10 min and 4-Chloroaniline (1.1mmol, 0.141 g) was added using a micro syringe as it is an irritant and the stirring is continued overnight at room temperature. The solution was turned to transparent. The worked up is carried out as mentioned in the above procedure[11] 3.1(a)

^{1}H NMR (300MHz, $CDCl_3$): δ_H 3.76 (s, 2H, CH_2), 7.10 (s, 1H, NH), 7.13-7.45(9H, m, ArH)

IR: vcm^{-1} 3276.470 (m, N-H Stretch), 1657.765 (s, C=O Stretch), 2926.227 (m, C-H Stretch), 648.713 (s, C-Cl Stretch) cm^{-1}

3.2 SYNTHESIS OF β-LACTAM ANTIBIOTICS

3.2(a) Preparation of Phenylacetic acid to 6-Aminopenicillanic acid



Beta-lactam Antibiotic 1

$C_{15}H_{18}O_4N_2S$

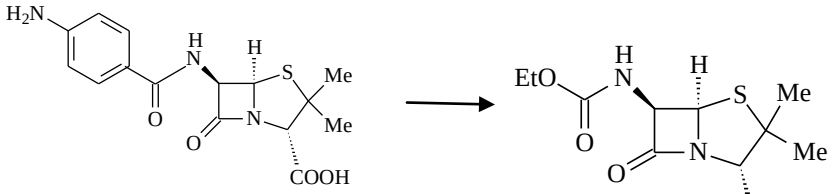
Phenylacetic acid (0.55 g, 4 mmol), triethylamine (0.55 mL, 4 mmol) was dissolved in dry DMF (5 mL). The reaction mixture is cooled to -10º C in ice/salt bath and ethyl chloroformate (0.38 mL, 4 mmol) was added drop wise to the reaction mixture was left to stir for 20 min followed by the addition of dry ice cold acetone (5 mL). A chilled solution was prepared previous day containing 6-APA (0.87g, 4 mmol), triethylamine (0.40 mL, 4 mmol) in water (10 mL) was added and the reaction is left to stir for 90 min and is allowed to return to room temperature.

The reaction mixture is quenched with the addition of water (5 mL) and the pH is adjusted to 7. The organic layer is washed with ethyl acetate (10 mL) and acidified to pH 2 and immediately extracted by ethyl acetate (3 x 10 mL). The ether layer is washed with water (2 x 10 mL) and extracted with 1M potassium bicarbonate solution which was then washed with ethyl acetate (2 x 10 mL) and freeze dried to give potassium salt of Penicillin. And the resulted mixture is evaporated under vacuum pressure. The obtained product was dissolved in methanol to remove the impurities with some amount of dry ether and it is evaporated again to get the pure form of penicillin derivative[12].

^{1}H NMR (300MHz, D_2O): δ_H 1.11-1.49 (m, 5H, ArH), 1.53 (s, 6H, CH_3), 2.09 (s, 2H, CH_2), 4.7 (s, 1H, β-lactam H) 5.25 (d, 1H, β-lactam H), 5.45 (d, 1H, β-lactam H), 8.05 (s, 1H, H).

IR: vcm^{-1}: 3371.059 (m, N-H Stretch), 1607.885 (s, C=O Stretch), 2978.515 (m, C-H Stretch), 1770.329 (s, β-lactam Stretch) cm^{-1}

3.2 (b) Attempt for the preparation of 4-Aminobenzoic acid to 6-Aminopenicillanic acid (Ethoxy Penicillin was formed)



Beta-lactam Antibiotic 3

$C_{15}H_{17}O_4N_3S$ **$C_{11}H_{15}O_5N_2S$**

4-Aminobenzoic acid (0.274 g, 2 mmol), triethylamine (0.20 mL, 2 mmol) was dissolved in dry DMF (2.5 mL). The reaction mixture is cooled to -10ºC in ice/salt bath and ethyl chloroformate (0.217 mL, 2 mmol) was added drop wise to the reaction mixture was left to stir for 20 min followed by the addition of dry ice cold acetone (2.5 mL). A chilled solution was prepared previous day containing 6-APA (0.433 g, 2 mmol), triethylamine (0.20 mL, 4 mmol) in water (5 mL) was added and the reaction is left to stir for 90 min and is allowed to return to room temperature[12].

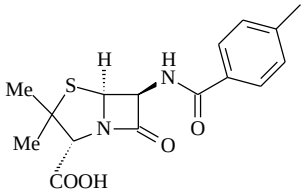
The reaction mixture is worked up as in procedure 3.2(a)

^{1}H NMR (300MHz, D_2O): δ_H 1.40 (s, 3H, CH_3), 1.51 (s, 6H, 2xCH_3), 4.13 (s, 2H, CH_2), 5.25 (d, 2H, H), 5.45 (d, 2H, H) 7.85 (s, NH, 1H).

IR: vcm^{-1} 3232.6 (m, N-H Stretch), 1602.4 (s, C=O Stretch), 1060.4 (s, C-O Stretch), 1766.4(s, β-lactam Stretch) cm^{-1}

LC-MS (ES^-): Calculated mass-287.077 formula ($C_{11}H_{15}N_2O_5S$) m/z 286.95 (100%, M-H) $^-$

3.2 (c) Preparation of p-Toluic acid to 6-Aminopenicillanic acid



Beta-lactam Antibiotic 5

$C_{16}H_{18}O_4N_2S$

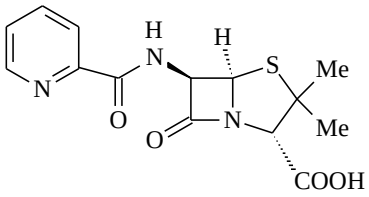
p - Toluic acid (0.273 g, 2mmol), triethylamine (0.20 mL, 2mmol) was dissolved in dry DMF (2.5 mL). The reaction mixture is cooled to -10ºC in ice/salt bath and ethyl chloroformate (0.217 mL, 2mmol) was added drop wise to the reaction mixture was left to stir for 20 min followed by the addition of dry ice cold acetone (2.5 mL). A chilled solution was prepared previous day containing 6-APA (0.433 g, 2 mmol), triethylamine (0.20 mL, 2 mmol) in water (5 mL) was added and the reaction is left to stir for 90 min and is allowed to return to room temperature.

The reaction mixture is worked up as in procedure 3.2 (a)

^{1}H NMR (300MHz, D_2O): δ_H 1.55 (s, 3H, CH_3), 2.26 (s, 3H, CH_3), 4.90 (d, 1H, β lactam-H), 5.20 (d, 1H, β lactam-H) 7.63-7.66 (m, 4H, ArH).

IR: vcm^{-1}: 3371.327 (m, N-H Stretch), 1673.921 (s, C=O Stretch), 2978.992 (m, C-H Stretch) 1770.493 (s, β-lactam Stretch) cm^{-1}

3.2 (d): Preparation of coupling of Nicotinic acid to 6-Aminopenicillanic acid



Beta-lactam Antibiotic - 6

$C_{14}H_{15}O_4N_3S$

Nicotinic acid (0.246g, 2mmol), triethylamine (0.20 mL, 2mmol) was dissolved in dry DMF (2.5 mL). The reaction mixture is cooled to -10ºC in ice/salt bath and ethyl chloroformate (0.217 mL, 2mmol) was added drop wise to the reaction mixture was left to stir for 20 min followed by the addition of dry ice cold acetone (2.5 mL).

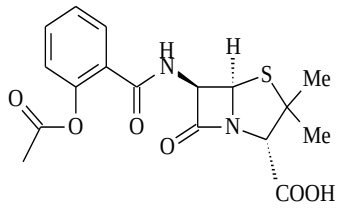
A chilled solution was prepared previous day containing 6-APA (0.433 g, 2 mmol), triethylamine (0.20 mL, 2 mmol) in water (5 mL) was added and the reaction is left to stir for 90 min and is allowed to return to room temperature[12].

The reaction mixture is worked up as in procedure 3.2 (a)

^{1}H NMR (300MHz, D_2O): δ_H 1.50-1.65 (m, 4H, ArH), 2.10 (s, 6H, CH_3), 4.10 (d, 1H, β lactam-H), 5.25 (d, 1H, β lactam-H), 5.45 (d, 1H, β lactam-H).

IR: vcm^{-1} 3321.112 (m, N-H Stretch), 1674.005 (s, C=O Stretch), 2970.549 (m, C-H Stretch), 1770.418 (s, β-lactam Stretch) cm^{-1}

3.2 (e) Preparation of coupling of Acetyl Salicylic acid (Aspirin) to 6-Aminopenicillanic acid



Beta-lactam Antibiotic - 7

$C_{17}H_{18}O_6N_2S$

Aspirin (0.316g, 2mmol), triethylamine (0.20 mL, 2mmol) was dissolved in dry DMF (2.5 mL). The reaction mixture is cooled to -10ºC in ice/salt bath and ethyl chloroformate (0.217 mL, 2 mmol) was added drop wise to the reaction mixture was left to stir for 20 min followed by the addition of dry ice cold acetone (2.5 mL).

A chilled solution was prepared previous day containing 6-APA (0.433g, 2mmol), triethylamine (0.20 mL, 2 mmol) in water (5 mL) was added and the reaction is left to stir for 90 min and is allowed to return to room temperature[12].

The reaction mixture is worked up as in procedure 3.2(a).

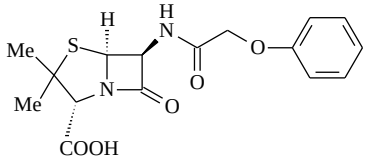
^{1}H NMR (300MHz, D_2O): δ_H 2.10 (s, 6H, CH_3), 4.10 (d, 1H, β lactam-H), 5.25 (d, 1H, β lactam-H), 5.45 (d, 1H, β lactam-H), 6.51-7.71 (m, 4H, ArH).

^{13}C NMR (75MHz, D_2O): δ_C 23.5422 ($C^{21}$and C^{22}), 13.9599 (C^1), 122.0344 (C^7), 181.5207 (C^{15}), 175.5806 (C^{11}), 76.0301 (C^{18}), 75.1720 (C^{16}), 67.1438(C^{19}), 144.0635-134.0861 (C^5- C^9)

IR: vcm^{-1}: 3282.723 (m, N-H Stretch), 1581.230 (s, C=O Stretch), 2965.211 (m, C-H Stretch), 1336.747 (s, C-O Stretch) cm^{-1}

LC-MS (ES^-): Calculated mass – 378.0885, molecular formula ($C_{17}H_{18}N_2O_6S$) m/z 377.00 (100%, $M-H)^-$.

3.2 (f) Preparation of Phenoxyacetic acid to 6-Aminopenicillanic acid



Beta-lactam Antibiotic - 12

$C_{16}H_{18}O_5N_2S$

Phenoxyacetic acid (0.304 g, 2 mmol), triethylamine (0.20 mL, 2 mmol) was dissolved in dry DMF (2.5 mL). The reaction mixture is cooled to -10ºC in an ice/salt bath and ethyl chloroformate (0.217 mL, 2 mmol) was added drop wise to the reaction mixture was left to stir for 20 min followed by the addition of dry ice cold acetone (2.5 mL). A chilled solution was prepared previous day containing 6-APA (0.433 g, 2 mmol), triethylamine (0.20 mL, 2 mmol) in water (5 mL) was added and the reaction is left to stir for 90 min and is allowed to return to room temperature.

The reaction mixture is worked up as in procedure 3.2(a)

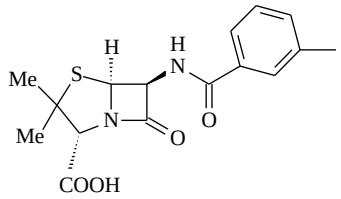
^{1}H NMR (300MHz, D_2O): δ_H 1.37 (s, 6H, β-lactam CH_3), 4.11 (s, 1H, β-lactam H), 4.35 (s, 2H CH_2)4.60 (s, 1H, β lactam-H), 5.38 (d, 1H, β lactam-H), 6.82-7.28 (m, 5H, ArH).

^{13}C NMR (75MHz, D_2O): δ_C 27.21 ($C^{19}$and C^{20}), 59.65 (C^{12}), 65.42 (C^{17}), 67.44 (C^8), 68.08 (C^{14}), 74.11 (C^{16}), 122.30 (C^4), 130.70 (C^5 and C^3), 158.91 (C^1), 171.98 (C^9), 174.75 (C^{13}) 74.11 (C^{16}).

IR: vcm^{-1}: 3370.447 (m, N-H Stretch), 1607.292 (s, C=O Stretch), 2965.651 (m, C-H Stretch), 1048.806 (s, C-O Stretch), 1769.903 (s, β-lactam Stretch) cm^{-1}

LC-MS (ES$^-$): Calculated mass - 350.093, molecular formula ($C_{16}H_{18}N_2O_5S$) m/z 348.95 (100%, M-H)$^-$.

3.2 (g) Preparation of m – Toluic acid to 6-Aminopenicillanic acid



Beta-lactam Antibiotic - 14

$C_{16}H_{18}O_5N_2S$

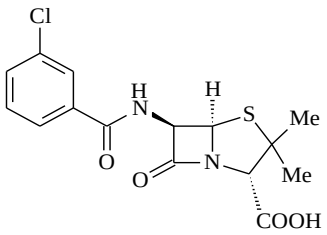
m-Toluic acid (0.272 g, 2 mmol), triethylamine (0.20 mL, 2 mmol) was dissolved in dry DMF (2.5 mL). The reaction mixture is cooled to -10ºC in ice/salt bath and iso-butyl chloroformate (0.273 mL, 2 mmol) was added drop wise to the reaction mixture was left to stir for 20 min followed by the addition of dry ice cold acetone (2.5 mL). A chilled solution was prepared previous day containing 6-APA (0.433 g, 2 mmol), triethylamine (0.20 mL, 2 mmol) in water (5ml) was added and stirred for 90 min and is allowed to return to room temperature. The reaction mixture is worked up as in procedure 3.2(a)

^{1}H NMR (300MHz, D_2O): δ_H 1.75 (s, 6H, β-lactam CH_3), 2.26 (s, 3H, CH_3), 5.10 (d, 1H, β-lactam H), 5.25 (d, 1H, β lactam-H), 7.20-7.70 (m, 4H, ArH).

IR: vcm^{-1}: 3322.035 (m, N-H Stretch), 1626.416 (s, C=O Stretch), 2929.366 (m, C-H Stretch), 1770.196 (s, β-lactam Stretch) cm^{-1}

LC-MS (ES$^-$): Calculated mass – 334.0984, molecular formula ($C_{16}H_{18}N_2O_4S$) m/z 333.00 (100%, M-H)$^-$.

3.2(h) Preparation of 3-Chlorobenzoic acid to 6-Aminopenicillanic acid via DCC method



Beta-lactam Antibiotic - 15

$C_{14}H_{15}O_4N_2SCl$

3-Chlorobenzoic acid (3.11 mmol, 0.485 g) and N-hydroxysuccinimide of (3.42 mmol, 0.394 g) was added and stirred with 20 mL of ethyl acetate at 0ºC and 0.706 g of DCC was added and is stirred overnight and allowed to cool at room temperature.

The resulted suspension was filtered through celite by making a bed of celite and filtrate is wash with ethyl acetate and subject for evaporation with rotary evaporation that afford to yield 3-Chlorobenzoic N-hydroxysuccinimide ester.

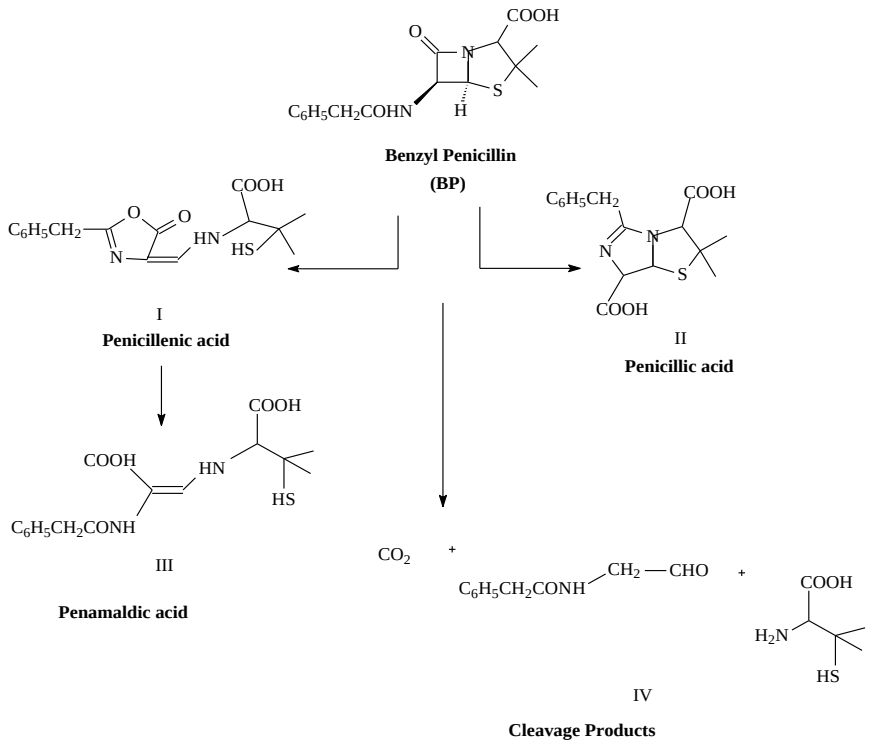
3-Chlorobenzoic-N-hydroxysuccinimide ester of (0.245 g, 1 mmol) is added and the flask is subjected to Nitrogen gas for drying for about 5 min. DMF is also dried using nitrogen gas using microlitre syringes and needles. 8 mL of DMF is added into the round bottom flask containing ester using syringe and finally 6 APA (1.1 mmol, 0.217 g) is added to the reaction mixture in presence of DMAP (0.12 g, 0.10 mmol) as a catalyst and the reaction mixture is stirred overnight at rt[11].

The resulting reaction mixture is worked up as in procedure 3.1(a).

IR: vcm^{-1}: 3322.149 (m, N-H Stretch), 1627.678 (s, C=O Stretch), 2930.351 (m, C-H Stretch), 1770.056 (s, β-lactam Stretch), 806.957 (s, C-Cl Stretch) cm^{-1}

The yield was not sufficient to analyze ^{1}HNMR, it gave noisy spectrum.

3.3 Hydrolysis of Benzyl Penicillin and β-lactam Antibiotic 12



β-lactam drugs undergo acid-catalysed hydrolysis when administered orally which inactivates the antibiotics. The hydrolysis pathway was explicated by scanning the antibiotic samples using UV-Vis Spectrophotometer. A stock solution of concentration $8x10^{-4}$ is prepared using Benzyl penicillin as a reference and pure moieties of synthesized β-lactam antibiotic derivative (β-lactam Antibiotic 12) and us diluted with 0.2 mol/dm^3 of HCl stock solution made up of concentrated Hydrochloric acid. Water is kept as a standard such that the instrument is balanced with the water sample. The samples are diluted to 1:1 ratio; the quartz cuvettes were washed and clean dried and to make sure that it doesn't create any imperfection in the optical path. Penicillin is dissolved in water, of antibiotic (1.5 mL) & HCl (1.5 mL) is added to check the absorbance. Care should be taken that as soon as the HCl is added the solution should be mixed using a pipette and the scanning should be done immediately. Three separate runs are made in different wavelengths at 322nm, 290nm and 240nm to record the rate of dissociation[16].

a. Calculation for Benzyl Penicillin at 240nm (Appendix 3.1a)

Slope (K) = -0.0041

4.10 E – 03

Total A=II + III

A= 0.977

AIII = 0.271

AII = 0.706

Moles II = 0.000108615

Mole fraction = 0.271538462

% Yield of the Final Product II = 27%

% Yield of the Final Product III = 4%

% Yield of the Final Product IV = 69%

b. Calculation for Benzyl Penicillin at 290nm (Appendix 3.1b)

Slope (K) = -0.0043

Slope = - 4.30E-03

AIII = 0.271

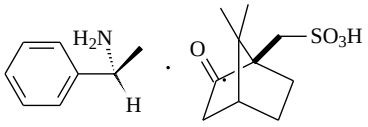
Mole Absorbtivity = 15800

Moles = 1.71519E-05

Moles fraction = 0.042879747

% Yield of the Final Product III = 4%

3.4 Preparation of racemic 2-phenylglycine with (1S)-(+)-camphor-10-sulfonic acid



D-PG **(+)-CS**

DL-Phenylglycine (DL-PG) (0.755 g, 5 mmol) was stirred with 8 mL of hot water and (1*S*) - (+)-camphor-10-sulfonic acid (1.275 g, 5.1 mmol) was stirred for about 10 min and the reaction mixture becomes clear. The solution was seeded with pulverized crystals of D-PG.(+)-CS and the mixture was stirred and cooled at room temperature with continuous stirring for 2h at 8º C.

The precipitated crystals was filtered and washed with small amount of cold water using Buchner apparatus and was dried at 80ºC for 5 h in a vacuum to get crude D-PG.(+)-CS.

Polarimerty: Measuring optical purity

Solution is prepared for polarimetry, D-PG (+) - CS (0.02 g, 0.0011 mmol) of into a 1 mL small volumetric flask and the volume was made up to the mark with 1M HCl.

The Polarimeter tube was rinsed with 1M HCl (solvent) and is filled with the same solvent and the polarimeter was calibrated to zero and the tube was dried in vacuum and the product solution was added using capillary pipette from the volumetric flask and the reading was noted[14].

Specific rotation can be calculated by the formula:

$$[\alpha]_D^{20} = \frac{\alpha D^{20}}{C/100 \times l}$$

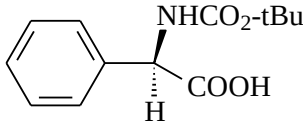
$$= \frac{-0.19}{2/100 \times 0.25}$$

C = concentration in g/1 mL

l = length of polarimeter tube in dm

= - 0.19 / 0.005 = **-38**

3.5 Conversion of (*R*)-2-Phenylglycine to its N-*tert*-Butoxycarbonyl (BOC) Derivative



$C_{13}H_{17}NO_4$

A 100 mL 3 necked round bottom flask, equipped with an efficient stirrer, a dropping funnel, reflux condenser and thermometer is charged with a solution (0.4 g, 0.001 mole) of NaOH in water (11 mL). Stirring was initiated L-Phenylglycine (+) CS (0.0001 mol, 0.384g) was added room

temperature and then diluted with tert-butyl alcohol (0.74 mL). To the well stirred clear solution of di-*tert*-butyl dicarbonate (0.218 mL, 0.001 mole) is added drop wise over 1 h.

A white precipitate appears during the addition of di-tert butyl dicarbonate. After a short induction period the temperature rises about 30-35º C. The reaction is brought to completion by further stirring overnight at room temperature. At this time, the clear solution will have reached a pH of 7.5-8.5.

The reaction mixture was extracted of pentane (2x250 mL) and the organic phase is extracted aqueous $NaHCO_3$ (3 x 100 mL). The combined aqueous layers are acidified with pH 1 – 1.5 by careful addition of 0.162g of $KHSO_4$ in 100 mL of water. The acidification was accompanied by copious evolution of CO_2.

The turbid mixture is then extracted with diethylether (4 x 40 mL). The combined organic layers was washed with water (2 x 200 mL), dried over $MgSO_4$. The solvent was removed under reduced pressure using. A transparent oil remains was treated with 150 mL of hexane and allowed to stand overnight.

Within one day the following portions of hexane is added with stirring to the partially crystallized product: 2x15 mL, 4x30 mL, 1x60 mL respectively. The solution was placed in a refrigerator overnight. The white precipitate was collected on a Buchner funnel and washed with cold pentane (20 mL).

The solid was dried under reduced pressure at ambient temperature to constant weight to give a 1st crop. The mother liquor is evaporated to dryness to leave yellowish oil, which was then treated with hexane (150 mL) and allowed to stand overnight.

Within one day the following portions of hexane was added with stirring to the partially crystallized product: 2x15 mL, 4x30, 1x60ml. The solution was placed in a refrigerator overnight. The white precipitate was collected on a Buchner funnel and washed with cold pentane. The solid was dried

under reduced pressure at ambient temperature to constant weight to give a 2nd crop to yield N-*tert*-Butoxycarbonyl-L-Phenylglycine[15]

% yield for the crop 1: **20%**

^{1}H NMR (300MHz, $CDCl_3$): δ_H 1.45 (s, 9H, ^{t}Bu), 5.37 (s, 1H, H), 7.39 (m, 5H, ArH), 7.41 (s, 1H, NH).

IR: vcm^{-1} 3377.308 (m, N-H Stretch), 1682.806 (s, C=O Stretch), 2981.109 (m, C-H Stretch), 1154.606 (s, C-O Stretch) cm^{-1}

4.0 CONCLUSION

There is eternal research on the discovery of novel antibiotic to overcome the antibiotic resistant strains of bacteria like Mycobacterium tuberculae. Objective of my research is to develop the pharmacodynamic property of the β-lactam drugs. The novel β-lactam antibiotic moieties which are synthesized were mainly via the mixed anhydride method using ethyl chloroformate or iso-butylchloroformate. A novel compounds (β-lactam antibiotic 7), (β-lactam antibiotic 12), (β-lactam antibiotic 14) moieties exhibit good result with ethylchloroformate and iso - butyl

chloroformate with a good yield. Which can be further studies need to be performed in this area of research by using techniques such as reverse phase HPLC to provide quantitative results.

Another novel compound β-lactam antibiotic 14 worked well with iso-butylchloroformate because the iso- butylchloroformate creates a steric hindrance to the carbonyl carbon and paves the way for nucleophilic attack on the carbonyl carbon of the chosen aromatic acid and thereby inhibiting the ethoxy contaminants.

DCC method was carried out using NHS as an activating reagent for the carboxylic acids, it fails to couple with amines/anilines due to the ability of reactivity of the anilines was difficult sequence of the reaction and this was mainly due to the reduced nucleophilicity of the aniline nitrogen. The poor reactivity of anilines is due to the increased conjugation of the lone pair of electrons present on the nitrogen molecule with the π-system of the aromatic ring. This was driven by the increased electron deficiency cause due to the electron withdrawal by the chlorine substituents on the aromatic ring.

But when DCC method was carried out in presence of DMAP as a nucleophilic catalyst, DMAP makes 6-APA more nucleophilic and thereby increasing the coupling activity.

I conclude that the method used for the synthesis of penicillin antibiotics can be carried out with different moieties of chloroformate to get better and purified β-lactam derivatives.

5.0 FUTURE WORK

Synthesised β lactam antibiotic moieties must be analysed by the reverse phase HPLC to check the purity of the synthesised antibiotic drug moieties. Activity of the synthesised compound must be tested by bioassay. The test for Zone of Inhibition has to be carried out on different strains of bacterial species to analyze the anti-bacterial activity of the synthesised β-lactam moieties. The mixed anhydride method used must be modified by using variety of chloroformates to yield pure products. DCC method can be used with any nucleophilic agent as a catalyst. The β-lactam antibiotic 7 can be used on hemorrhage cell lines to check the antiplatelet activity of the synthesised

moiety. In future the synthesised penicillins are deprotected by BOC derivatives. HCl deprotection or TFA deprotection must be carried out to protect the amide group on the β-lactam scaffold for the further peptide synthesis.

6.0 REFERENCES

1. Fischbach, M.A., Walsh. C.T., Antibiotics of Emerging Pathogens, Science, 2009, Vol.325, p 1089-1093.
2. An Introduction to Medicinal Chemistry (2nd Edition) by Graham L. Patrick
3. www.scripps.edu/chem/nicalaou/images/sample chapter13.pdf cited on 20/04/2010.
4. Donald J. Tipper and Jack L. Strominger, Mechanism of action of Penicillins: A proposal based on their structural similarity to Acyl-D-alanyl-D-alanine, 1965 Vol.54, No.4, pp 1133-1141.

5. www.sciencemag.org/content/vol 278/ issue 5344/ images/ medium/ 1731-b-1-med.gif
6. www.mikeblaber.org/oldwine/BCH4053/lecture15/lecture15/html.
7. Indrani Banik, Frederick F. Becker and Bimal K. Banil, Stereoselective synthesis of β-lactams with Polyaromatic Imines: Entry to New and Novel Anticancer Agents, J. Med.Chem.2003, Vol.46, p.12-15.
8. G.N Rolinson, A.M. Geddes, The 50th anniversary of the discovery of 6-aminopenicillanic acid (6-APA), International journal of Antimicrobial agents Vol. 29 (2007) p.3-8.
9. Bimal K. Banik, Frederick F. Becker And Indrani Banik, Synthesis of anticancer β-lactams: mechanism of action, Bioorganic and Medicinal Chemistry 12 (2004) p.2553-2528.
10. Bimal K. Banik, Indrani Banik and Frederick F. Becker, Stereocontrolled synthesis of anticancer β-lactams via the Staudinger reaction, Bioorganic and Medicinal Chemistry 13 (2005) p. 3611-3622.
11. Penicillin project for MSc students; Newcastle University.
12. Bertil Ekstrom; Gomez-Rivella, A; Mullberg. R; Thelin H,; Sjoberg B., Semisynthetic Penicillins, Acta Chem.Scand. 1965, Vol. 19, p. 281 - 299.
13. W.Clark. Still, Michael Kahn and Abhijit Mitra, Rapid chromatographic technique for preparative separations with moderate resolution. J.Org.Chem, Vol 43, No. 14, 1978.
14. Ryuzo Yoshika, *Hajime Hiramatsu, **Kimio Okamura, Ikuko Tsujioka and Shin-ichi Yamada. Crystal structure solubility relationships in Optical resolution by diastereomeric salt formation of DL-PG with (1*S*)-(+)-Camphor-10-sulfonic acid, J. Chem. Soc., Perkin Trans 2, 2000, 2121-2128.
15. Keller, O., Keller, W.E., van Look, G., Wersin, G., tert-Butoxycarbonylation of amino acids and their derivatives, Org. Synth. 1990, Coll. Vol.7, 70.
16. McCarrick, T.A; McLafferty, F.W., Penicillin Hydrolysis: a kinetic study of multistep, multiproduct reaction, J.Chem.Educ 1984, 61, 463-464.